Supporting Information for:

Using EPR to compare PEG-*branch*-nitroxide "bivalent-brush polymers" and traditional PEG bottle-brush polymers: branching makes a difference

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Materials and Methods.

All reagents and solvents were purchased from Aldrich or VWR and used as supplied unless otherwise noted. Ruthenium catalyst **1**,[1] norbornene-PEG-*branch*-alkyne MM (**8**),[4] 4-azido-2,2,6,6,-tetramethyl-1-piperidinyloxy free radical (**9**)[5] and **Q**[6] were prepared according to literature procedures. Degassed dichloromethane (DCM), tetrahydrofuran (THF) and dimethyl sulfoxide (DMSO) were passed through solvent purification columns prior to use.[7]

Liquid chromatography–mass spectrometry (LC/MS) and preparative HPLC were performed on an Agilent 1260 LC system equipped with a Zorbax SB-C18 rapid resolution HT column and a Zorbax SB-C18 semi-preparative column. Solvent gradients consisted of mixtures of nano-pure water with 0.1% acetic acid (AcOH) and HPLC-grade acetonitrile. Mass spectra were obtained using an Agilent 6130 single quadrupole mass spectrometer.

Dynamic light scattering (DLS) measurements were made at room temperature using a Brookhaven ZetaPALS DLS instrument. Samples were dissolved in nanopure water at a concentration of ~1 mg / mL. A fresh, clean, polystyrene cuvette was washed with compressed air to remove dust. The sample solution was passed through a 0.4 μm Teflon syringe filter directly into the cuvette; the cuvette was capped and placed in the DLS instrument for particle sizing. At least 3 measurements were made per sample and average hydrodynamic diameters were calculated by fitting the DLS correlation function using the CONTIN routine (ISDA software package from Brookhaven instruments).

^{1}H nuclear magnetic resonance (^{1}H-NMR) and ^{13}C nuclear magnetic resonance (^{13}C-NMR) spectra were recorded on Bruker AVANCE-400 NMR spectrometers in the Department of Chemistry Instrumentation Facility at MIT. Chemical shifts are expressed in parts per million (ppm), and splitting patterns are designated as s (singlet), d (doublet), m (multiplet) and br (broad). Coupling constants *J* are reported in Hertz (Hz). Nuclear magnetic resonance (NMR) experiments were performed on either a Mercury 300 MHz spectrometer or an INOVA 500 MHz spectrometer. MestReNove NMR 7.0.1 software was used to analyze the NMR spectra.

Gel permeation chromatography (GPC) measurements were performed on an Agilent 1260 LC system with two Shodex KD-806M GPC columns in series at 60 °C and a flow rate of 1 mL / min. Dimethyl formamide (DMF) with 0.1M LiBr was used as the eluent. A T-rEX refractive index detector (Wyatt) and a DAWN EOS 18 angle light scattering (MALS) detector (Wyatt) were used for polymer analysis.

High-resolution mass spectrometry (HRMS) was obtained using a Bruker Daltonics APEXIV 4.7 Tesla Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (FT-ICR-MS). Matrix assisted laser desorption ionization mass spectrometry (MALDI) measurements were performed using a Voyager De_Pro TOF mass spectrometer (Applied Biosystems) outfitted with a 355 nm YAG laser from Blue Ion Technologies. In a typical experiment, 1.0 mg of polymer sample was dissolved in 100 ∫L of THF and diluted 10-fold with the MALDI matrix, dithranol (10 mg / mL in THF). To each sample was added 0.1 ∫L of saturated NaI in ethanol and 0.35 μL of the sample-matrix mixture S3 was spotted on a MALDI plate for analysis. The Voyager De_Pro was operated in reflectron mode with an accelerating voltage of 20,000 V, grid voltage of 95.2%, guide wire 0.03%, extraction delay time 250 ns, acquisition mass range 800-5000 Da, and laser rep rate 20 Hz. The instrument was calibrated externally using a Sequazyme Mass Standard Kit supplied by Applied Biosystems.

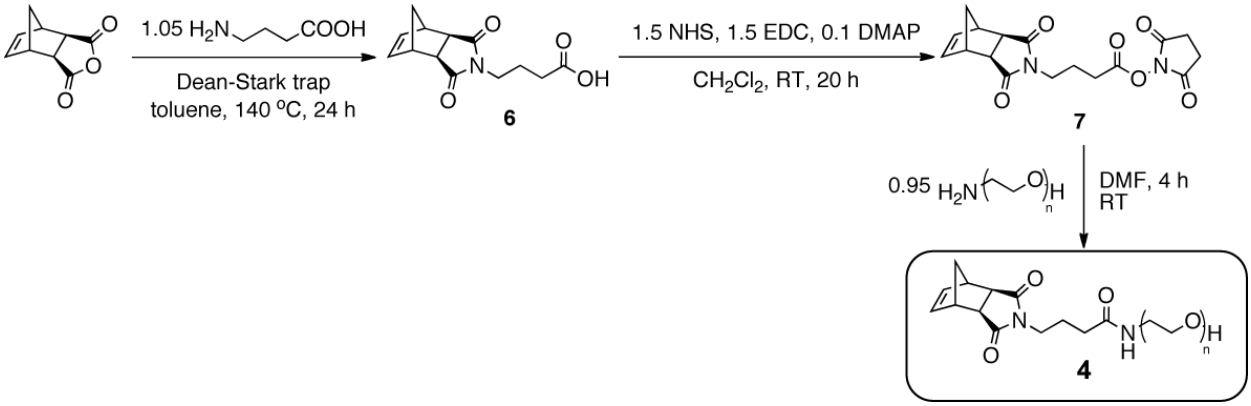
Photolysis experiments were performed using a Multiple Ray Lamp (UVP) fitted with an 8 W, longwave, filtered blacklight bulb (365 nm). Sample vials were placed as close as possible to the light source and irradiated for the desired time before analysis by DLS.

EPR spectroscopy was performed on a Bruker EMX X-band spectrometer. EPR tubes with O. D. 1 mm were used. Typical parameters used for the EPR measurements are modulation frequency: 100 KHz; modulation amplitude: 1 G; time constant: 11 ms; conversion time: 86 ms; scan time: 86 s; number of scans: 5. For the quenching experiments, the solutions were prepared in air. The EPR spectra were recorded following addition of the quencher. The peak intensity of the low field peak was used for calculation of the percentage of quenching.

TEM images were obtained at the MIT Center for Materials Science and Engineering on a JEOL 200CX TEM instrument equipped with a 1k x 1k CCD camera. The samples were prepared as follows: 5.0 μL of a 0.050 mg/mL solution of 20xL (or 15 xL) BASP polymer was deposited via pipet on top of a carbon film-coated 200-mesh copper grid (purchased from Electron Microscopy Sciences) placed on a piece of parafilm carbon-coated side up. The sample was allowed to dry at ~40^{o}C, and then the grid was placed carbon-coated side up in a glass insert for 2-mL vials. The insert was then placed inside a 3-mL scintillation vial, and to the vial (outside the insert) was added 0.50 mL of a 4% $OsO_{4(aq)}$; the vial was capped and allowed to stand overnight. The grid was then removed and was ready for TEM imaging.

Computation of EPR spectra. The computation of the EPR spectra was performed according to a well-known procedure.[8] The computation allowed us to extract the correlation time for the rotational motion, τ. An increase of this parameter indicates an increased strength of interaction of the probe with its environment. We assumed a Brownian rotational diffusional motion with $\tau = 1/(6D)$, where D is the diffusion coefficient. According to the diffusional model and probe geometry, the main mobility parameter is the perpendicular component of the correlation time, τ_{perp}. The accuracy of the spectral computation for this parameter was ± 0.01 ns.

Synthesis of Novel Compounds.

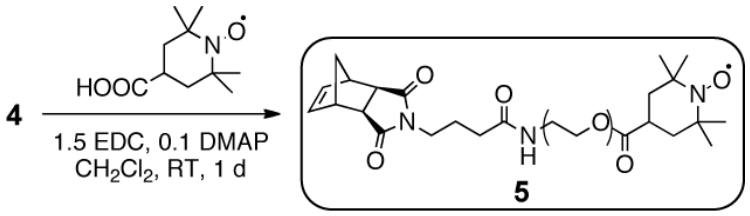


Scheme S1. Synthesis of MM **4**.

Norbornene-butyric acid 6. A solution of *gamma*-aminobutyric acid (1.0 g, 6.10 mmol) and *cis*-5-norbornene-*exo*-2,3-dicarboxylic anhydride (660 mg, 6.40 mmol) in toluene (30 mL) was added to a dried, 150 mL round-bottom flask fitted with a Dean-Stark trap. The flask was immersed in an oil bath preset to 140 °C and stirred for 24 h under nitrogen atmosphere. After this time, the solution was transferred to a separatory funnel and washed 3 times with 1M HCl, once with brine, and dried over magnesium sulfate. Removal of toluene by rotary evaporation yielded **4** (987 mg, 65%) as a faint white solid. ^{1}H NMR (500 MHz, $CDCl_3$): ™ 10.70 (s, 1H), 6.14 (s, 2H), 3.39 (t, J = 7.1 Hz, 2H), 3.11 (s, 2H), 2.56 (d, J = 0.8 Hz, 2H), 2.22 (t, J = 7.5 Hz, 2H), 1.77 – 1.69 (m, J = 7.4 Hz, 2H), 1.36 (d, J = 9.9 Hz, 1H), 1.06 (d, J = 9.8 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$): ™ 178.18, 177.48, 137.76, 134.41, 128.88, 128.08, 125.17, 47.70, 45.02, 42.67, 37.69, 31.20, 22.76. TOF HRMS: calcd. for $C_{13}H_{15}NO_4$ $[M - H]^-$, 248.0928; found, 248.0921.

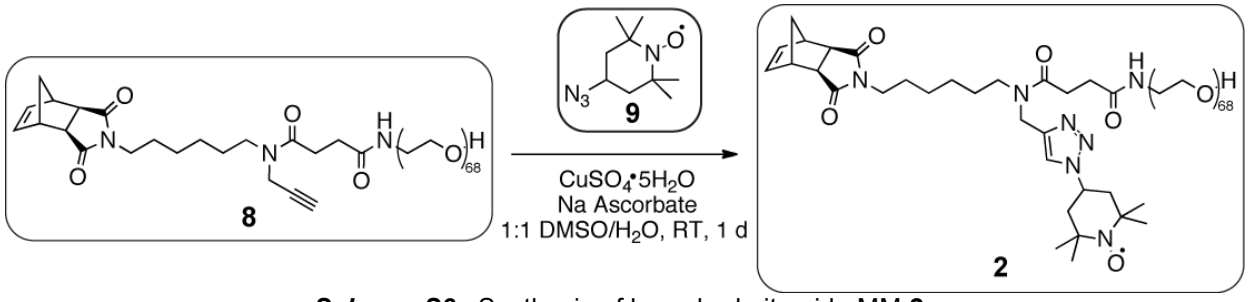
Norbornene-*N*-hydroxysuccinimidyl (NHS)-ester 7. DCM (33 mL) was added to a flask containing *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC, 310 mg, 1.50 mmol), 4-(dimethylamino)pyridine (DMAP, 12 mg, 0.10 mmol) and **6** (249 mg, 1.00 mmol). The resulting solution was stirred for 10 minutes. *N*-Hydroxysuccinimide (NHS, 171 mg, 1.50 mmol) was added to the flask and the resulting solution was stirred at RT for 20 hrs. The solution was filtered over celite and concentrated by rotary evaporation. The crude product was purified by silica gel column chromatography (50% EtOAc/hexanes, TLC R_f = 0.16) to yield **7** (150 mg, 43%) as a white-solid. ^{1}H NMR (500 MHz, $CDCl_3$): ™ 6.20 (s, 2H), 3.48 (t, J = 7.1 Hz, 2H), 3.18 (s, 2H), 2.74 (s, 4H), 2.61 (d, J = 1.2 Hz, 2H), 2.59 – 2.53 (m, 2H), 1.94 – 1.87 (m, J = 18.2, 7.4 Hz, 2H), 1.43 (d, 1H), 1.10 (d, J = 9.9 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): ™ 178.15, 169.21, 167.95, 138.02, 48.05, 45.38, 43.07, 37.66, 28.82, 25.79, 23.06. TOF HRMS: calcd. for $C_{17}H_{18}N_2O_6$ $[M + Na]^+$, 369.10629; found, 369.10468.

Norbornene-PEG macromonomer 4. *O*-(2-Aminoethyl)poly(ethylene glycol) (500 mg, 0.17 mmol) and **7** (60.55 mg, 0.18 mmol) were dissolved in anhydrous DMF (588 mL) and the resulting solution was stirred at room temperature for 4 hrs. The reaction mixture was then added dropwise to diethyl ether (30 mL) to precipitate **4** as a white solid. MM **4** was isolated by centrifugation and decanting off the ether before redissolving in DCM (500 mL). This process of precipitation, centrifugation and re-dissolution was repeated five times. After the fifth iteration, the precipitate was dried under vacuum to afford macromonomer **4** (378 mg, 70%) as a white powder. NMR and MALDI mass spectrum are shown in Figures S1 and S2, respectively.



Scheme S2. Synthesis of nitroxide terminated MM **5**.

Norbornene-PEG3k-TEMPO telechelic MM 5. DCM (1 mL) was added to a vial containing **4** (100 mg, 30.3 μmol), EDCI (8.7 mg, 45.5 μmol), DMAP (~0.4 mg, 3 μmol), and 4-carboxy-TEMPO (9 mg, 45.5 μmol). The solution was stirred at RT for 1 d. The reaction mixture was concentrated by rotary evaporation, dissolved in H_2O, and purified by preparative HPLC (linear gradient of 95:5 water-0.1% AcOH:MeCN to 5:95 water-0.1% AcOH-MeCN over 12 min). The fractions containing pure MM were combined and concentrated on a rotary evaporator. The resulting residue was dissolved in DCM, dried over Na_2SO_4, filtered, and dried under vacuum to give pure **5**. The ^{1}H NMR spectrum was difficult to interpret due to broadening from the TEMPO free radical. The MALDI spectrum for **5** is shown in Figure S3.



Scheme S3. Synthesis of branched nitroxide MM **2**.

Norbornene-PEG-*branch*-TEMPO MM 2. TEMPO-azide **9** (1.01 equiv. to alkyne) was combined with norbornene-PEG-*branch*-alkyne **8** (100 mg, 29.4 μmol) in a 2 mL HPLC vial and 1:1 DMSO:H_2O (0.5 mL) was added. A spatula tip of sodium ascorbate was added followed by a 1.0 M solution of $CuSO_4$ in H_2O (88 mL, 3 equiv. to alkyne). The mixture was flushed with argon, sealed with a septum, and stirred until completion (as monitored by LC-MS) which was typically ~1 h. After the required time, the TEMPO-loaded MM was purified by preparative HPLC (linear gradient of 95:5 water-0.1% AcOH:MeCN to 5:95 water-0.1% AcOH-MeCN over 12 min). The fractions containing pure MM were combined and concentrated on a rotary evaporator. The resulting residue was dissolved in DCM, dried over Na_2SO_4, filtered, and dried under vacuum to give pure **2**. The ^{1}H NMR spectrum was difficult to interpret due to broadening from the TEMPO free radical. The MALDI spectrum for **2** is shown in Figure S4.

General ROMP Methods. *All ROMP reactions were performed in a glovebox under N_2 atmosphere.* Stock solutions of the various monomers (**2**, **3**, **4**, and **5**) were prepared in CH_2Cl_2 (0.1 M). A stock solution of catalyst **1** was prepared in CH_2Cl_2 (1 mg / mL). The first monomer to be polymerized was transferred to a vial with a stir bar. Catalyst **1** was added such that the ratio of monomer to **1** was as desired. The reaction was allowed to proceed for 30 min before addition of the next monomer as necessary. After addition of each new monomer the reaction was allowed to proceed for 30 min. After the desired number of monomers were polymerized, the reaction vial was removed from the glovebox, quenched with ethyl vinyl ether (2 drops), and concentrated under vacuum.

Spectral Data.

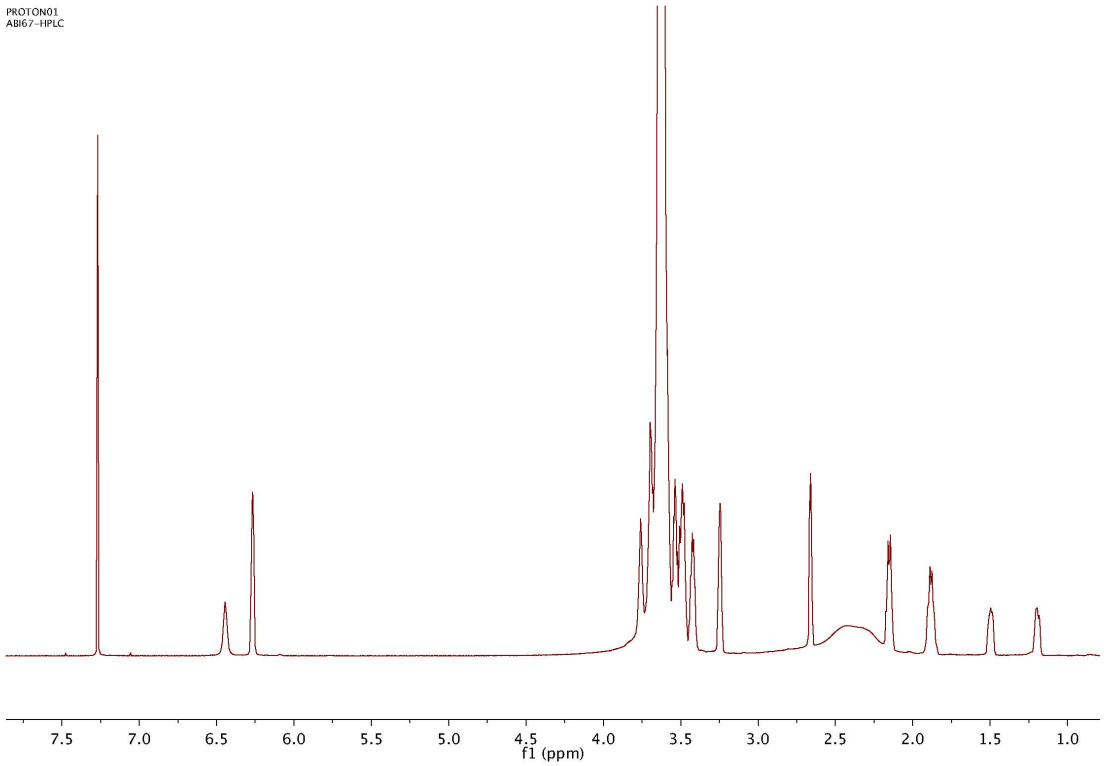


Figure S1. ^{1}H NMR spectrum of MM **4** in $CDCl_3$.

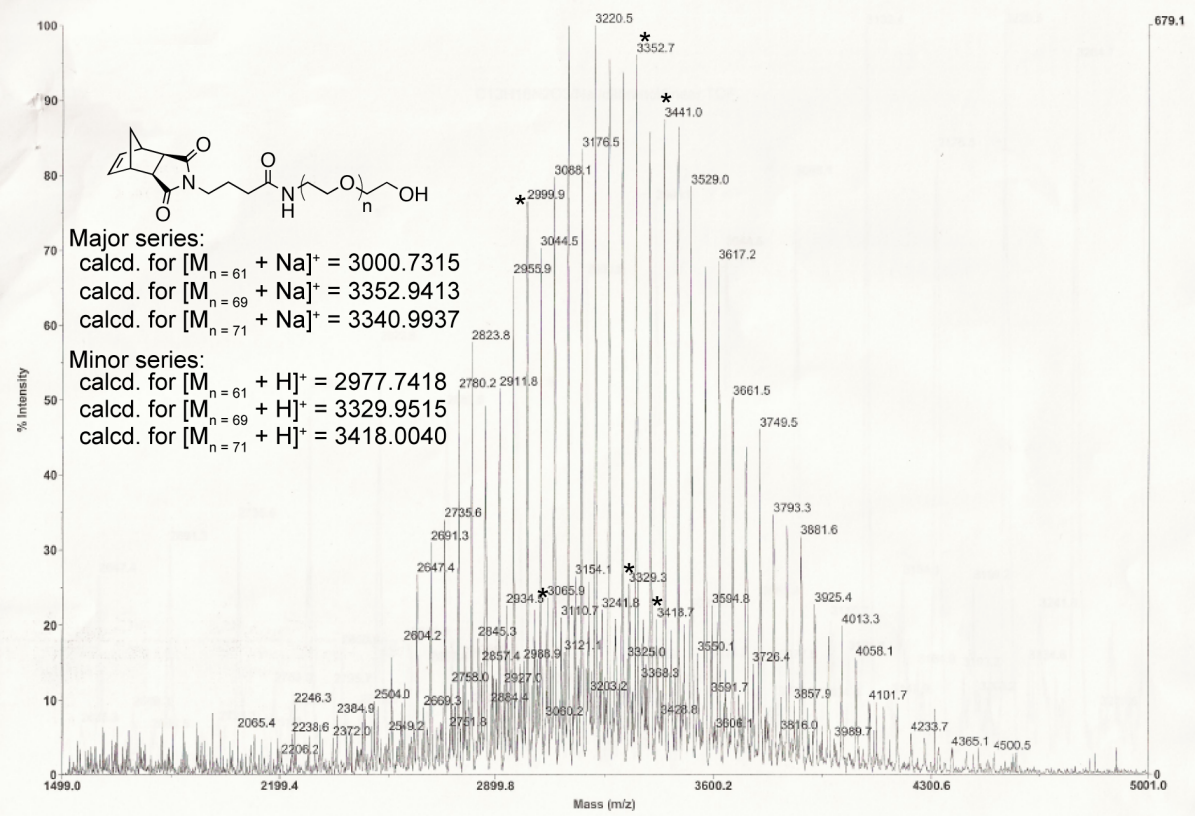


Figure S2. MALDI spectrum of MM **4**.

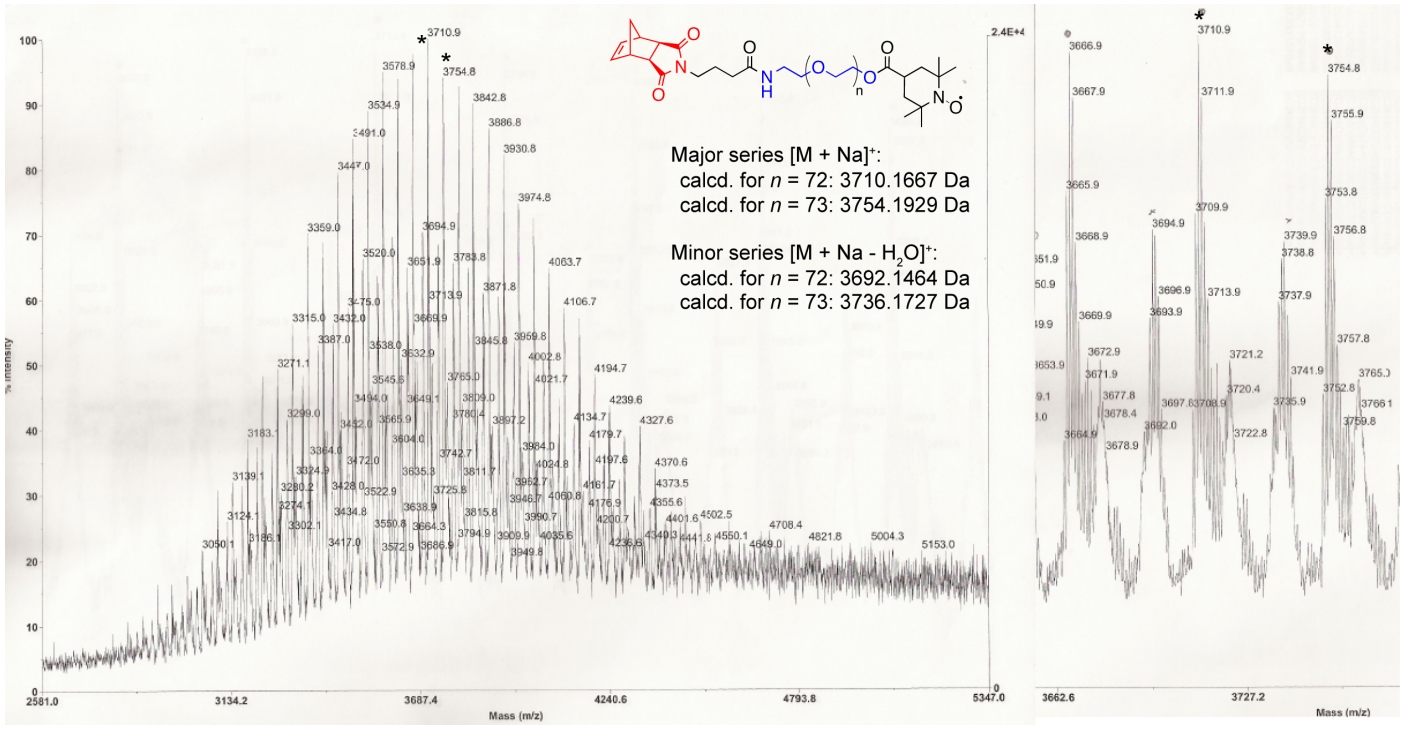


Figure S3. MALDI spectrum for MM **5**.

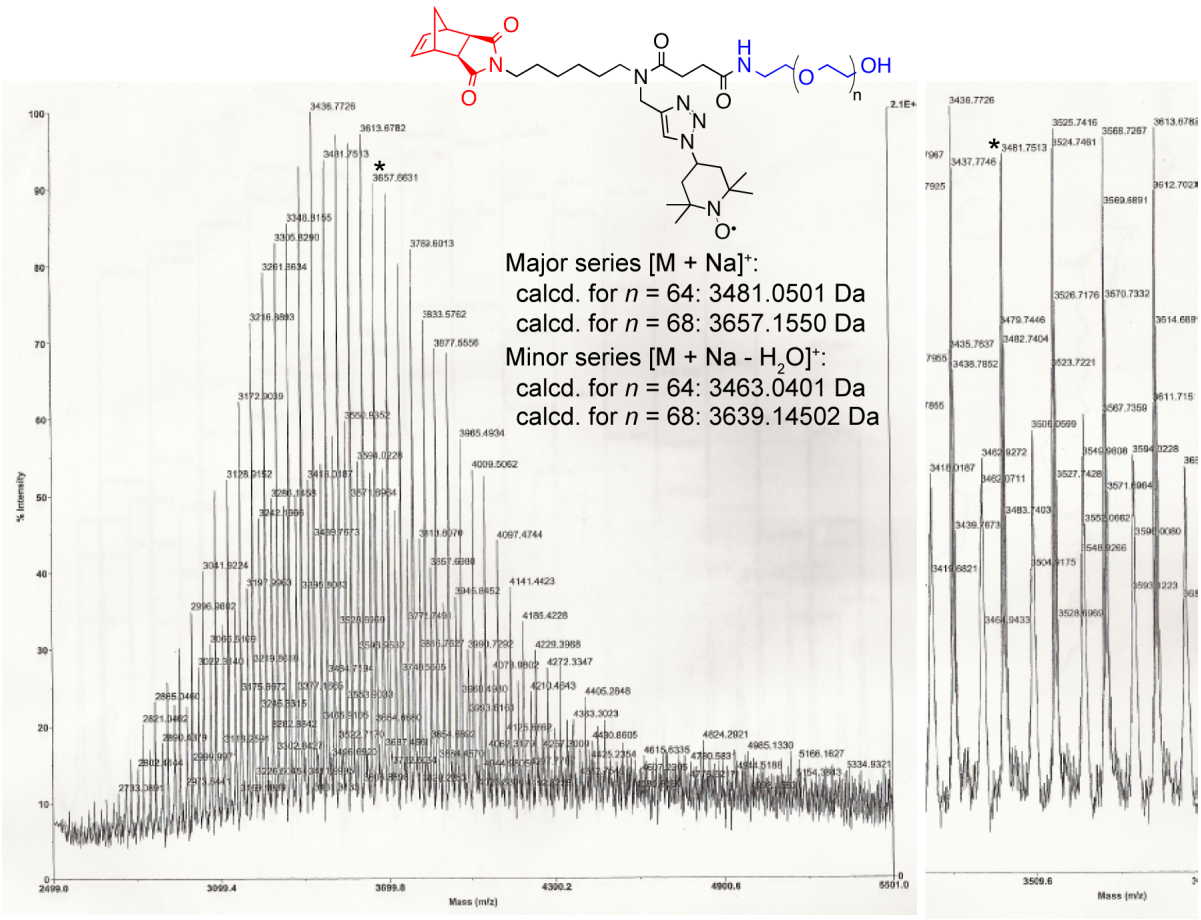


Figure S9. MALDI spectrum for MM **2**.

Supplemental References.

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